Resveratrol and novel potent activators of SIRT1: effects on aging and age-related diseases

Mitchell D Knutson and Christiaan Leeuwenburgh

Studies show that the plant polyphenol resveratrol can extend the lifespan of yeast, worms, flies, and fish. It also mitigates the metabolic dysfunction of mice fed high-fat diets. Resveratrol appears to mediate these effects partly by activating SIRT1, a deacetylase enzyme that regulates the activity of several transcriptional factors and enzymes responsive to nutrient availability. However, few foods contain resveratrol and humans metabolize it extensively, resulting in very low systemic bioavailability. Substantial research effort now focuses on identifying and testing more bioavailable and potent activators of SIRT1 for use as pharmacologic interventions in aging and age-related disorders.

© 2008 International Life Sciences Institute

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenol that belongs to the stilbene family of phytoalexins, which are antibiotic compounds produced by plants in response to infection. Resveratrol has been detected in at least 72 plant species but is present in only a limited number of common foods. Grapes, grape juice, red wine, and peanuts represent the richest dietary sources of resveratrol. Cranberries, blueberries, and tomato skin also contain this polyphenol, though at levels <10% of those reported for grapes. Dietary resveratrol exists as the free trans-or cis-isomer or conjugated with glucose (known as resveratrol glucoside or piceid). Although trans-resveratrol is absorbed efficiently by humans, the gut and liver metabolize it extensively, resulting in exceedingly low systemic bioavailability.

Resveratrol has generated intense scientific and public interest in recent years, mainly because of its widely reported ability to delay aging and prevent age-related diseases. As a result, a multitude of different resveratrol supplements have now appeared on the market. A cross-sectional study found that supplemental resveratrol is taken by two-thirds of individuals who routinely consume multiple dietary supplements. The salutary effects of resveratrol were originally thought to derive from its antioxidant properties. Indeed, the high concentration of resveratrol in red wine is frequently cited to account for the “French paradox,” the observation that the French have relatively low rates of cardiovascular disease despite consuming diets rich in saturated fat. Recent research, however, is converging on a different molecular mechanism that underlies the pleiotropic effects of this compound. Studies in a variety of species indicate that resveratrol seems to exert benefit by activating SIRT1, a member of the sirtuin family of nicotinamide adenine dinucleotide (NAD+)-dependent deacetylases. Much research effort is now directed at identifying more potent, and more bioavailable, activators of SIRT1 in an effort to combat aging and associated diseases.

SIRT1, AGING, AND THE ROAD TO RESVERATROL

Sirtuin 1 (SIRT1) is an enzyme that removes acetyl groups from specific proteins (Figure 1). Acetylation of lysine residues is a frequent post-translational modification affecting protein activity and stability. Deacetylation of nuclear proteins, such as histones, plays a major role in regulating gene expression. SIRT1 was first identified as the human orthologue of yeast Sir2p (silent information

Affiliations: MD Knutson is Assistant Professor with the Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida, USA. C Leeuwenburgh is Professor and Chief, Biology ofAging, with the Department ofAging and Geriatrics, College ofMedicine, University of Florida, Gainesville, Florida, USA.

Correspondence: MD Knutson, Food Science and Human Nutrition Department, PO Box 110370, University of Florida, Gainesville, FL, USA.
E-mail: mdknutson@ifas.ufl.edu; Phone: +1-352-392-1991 ext. 204; Fax: +1-352-392-9467.

Key words: life span, polyphenol, red wine, sirtuin


Nutrition Reviews® Vol. 66(10):591–596

591
regulator2protein), an NAD\(^+\)-dependent histone deacetylase involved in chromatin silencing. Structural similarities to yeast Sir2p led to the initial characterization of human SIRT1 as a histone deacetylase, but a number of nonhistone targets of SIRT1 have been identified as well. Nonhistone SIRT1 substrates include cytosolic acetyl-CoA synthetase\(^9\), involved in fatty acid synthesis, and a variety of transcription factors that mediate cellular responses to fasting,\(^{10}\) insulin,\(^{11}\) and inflammation.\(^{12}\)

Studies in the yeast Saccharomyces cerevisiae established a link between SIR2 (the gene encoding Sir2p) and aging by demonstrating that SIR2 mutants had shorter life spans and that increasing SIR2 dosage extended life span.\(^{13}\) In a follow-up study, the decreased longevity of the SIR2 mutants could not be increased by caloric restriction,\(^{14}\) the only dietary intervention that consistently extends life and health span in all organisms studied to date. This observation directly implicated Sir2p – and by extension SIRT1 – in mediating the positive biological effects of calorie restriction. Efforts were next directed at identifying compounds that activated SIRT1. By screening a number of small molecule libraries, Howitz et al.\(^{15}\) identified a structurally related group of polyphenolic compounds effective at stimulating recombinant SIRT1 activity. The group included the plant polyphenols resveratrol, butein, piceatannol, and quercetin. Of these compounds, resveratrol proved most potent, increasing the catalytic rate of SIRT1 in vitro by 13-fold. Resveratrol also increased the activity of recombinant yeast Sir2p. Importantly, treatment of S. cerevisiae with resveratrol increased average life span by 70% and significantly increased maximum life span. Since these studies in yeast, resveratrol has proven effective at increasing the life span of nematode worms (Caenorhabditis elegans), flies (Drosophila melanogaster),\(^{16}\) and a short-lived species of fish (Nothobranchius furzeri).\(^{17}\)

### EFFECTS OF RESVERATROL AND SIRT1 ACTIVATION IN ANIMAL STUDIES

The first indication that resveratrol could extend life span in mammals, as it did in lower organisms, was provided by Bauer et al.,\(^{18}\) who studied middle-aged (1-year-old) mice fed high-calorie diets (60% of calories from fat). High-fat diets are well known to induce obesity, triggering inflammatory states and comorbidities, such as diabetes and atherosclerosis, which decrease life span. To determine if resveratrol exhibited positive effects on life span under this dietary condition, mice were fed either a standard diet or a high-fat diet with or without resveratrol. The resveratrol diet provided an average of 22.4 mg resveratrol/kg/day, which is a feasible daily dose for humans. Although both groups of mice consuming the high-fat diet became obese, the ones receiving resveratrol lived longer, having survival curves similar to control animals. Moreover, the resveratrol-treated mice displayed enhanced insulin sensitivity and increased hepatic mitochondrial numbers similar to calorie-restricted animals with greater SIRT1 expression.\(^{19}\)

An important remaining question is whether resveratrol can extend life span in animals fed a standard diet. One recent study\(^{20}\) found that mice consuming a standard diet did not live longer when supplemented with resveratrol starting at 1 year of age, although it did increase the average life span of mice fed a high-fat diet, as observed previously.\(^{18}\)

Evidence is now rapidly emerging showing positive effects of resveratrol and SIRT1 activation on several age-related disorders including type 2 diabetes, cardiovascular disease, neurodegeneration, and inflammation. Table 1 provides a summary of selected studies published within the last 2 years.\(^{18,20–29}\) SIRT1 activation most likely mediates the therapeutic effect of resveratrol, at least in diet-induced obesity, because transgenic overexpression of Sirt1 in mice recapitulates many of the positive metabolic effects of dietary resveratrol.\(^{25}\) As with resveratrol, future
studies need to determine if SIRT1 overexpression increases life span in mammals. Overexpression of heart-specific SIRT1, though cardioprotective, did not extend the life span of mice fed a standard diet.\(^ {23} \)

### NOVEL POTENT ACTIVATORS OF SIRT1

A recent study by Milne et al.\(^ {22} \) used a high-throughput screening method to identify novel potent activators of human SIRT1. The chemical screen was performed by Sirtris Pharmaceuticals, a biotechnology company focused on discovering and developing drugs to treat diseases of aging. In total, they screened 290,000 small molecules and identified 127 candidate compounds. Three of these small molecule activators – named SRT1460, SRT1720, and SRT2183 – were synthesized for further study. The compounds are structurally related to each other, but notably distinct from resveratrol. Potency of each compound was initially assessed in vitro by determining the maximum activation as well as the concentration at which the compounds increased SIRT1 enzyme activity by 50% (EC\(_{50}\)). All compounds were compared to resveratrol, which displayed an EC\(_{50}\) of 46 \(\mu\)M and maximum activation of 201%. SRT1460, SRT2183, and SRT1720 were effective at much lower concentrations than resveratrol, exhibiting EC\(_{50}\) values of 2.9, 0.36, and 0.16 \(\mu\)M, respectively. SRT1720 maximally activated SIRT1 activity by 780%, nearly four times more than resveratrol, whereas SRT1460 and SRT2183 achieved a maximum activation of 447% and 296%. The ability of these compounds to activate endogenous SIRT1 function was investigated by using an osteosarcoma cell line (U2OS cells) and an in-cell Western assay to measure the extent of deacetylation of p53, a known substrate of SIRT1.\(^ {26} \)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Animal model</th>
<th>SIRT1 modulation</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2 diabetes</td>
<td>DIO mouse</td>
<td>Dietary resveratrol</td>
<td>Increased insulin sensitivity, decreased IGF-1</td>
</tr>
<tr>
<td></td>
<td>Baur et al. (2006)(^ {18} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIO mouse</td>
<td>Dietary resveratrol</td>
<td>Increased insulin sensitivity, increased aerobic capacity</td>
</tr>
<tr>
<td></td>
<td>Lagouge et al. (2006)(^ {21} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIO, ob/ob mouse, fa/fa rat</td>
<td>Gavage resveratrol, SRT1720, SRT501</td>
<td>Increased insulin sensitivity, decreased blood glucose</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>Transgenic mouse</td>
<td>Heart-specific Sirt1 overexpression</td>
<td>Decreased cardiac hypertrophy, apoptosis and dysfunction</td>
</tr>
<tr>
<td></td>
<td>Alcendor et al. (2007)(^ {21} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conditional knockout mouse</td>
<td>Sirt1 deletion in vascular endothelial cells</td>
<td>Blunting of ischemia-induced neovascularization</td>
</tr>
<tr>
<td></td>
<td>Potente et al. (2007)(^ {24} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>apo E(^ {−/−} ) mouse</td>
<td>Dietary resveratrol</td>
<td>Increased plasma HDL-C, decreased plasma LDL-C</td>
</tr>
<tr>
<td></td>
<td>Do et al. (2008)(^ {25} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurodegeneration</td>
<td>p25 transgenic mouse</td>
<td>ICV resveratrol injection, Sirt1 lentivirus</td>
<td>Protection against neurodegeneration</td>
</tr>
<tr>
<td></td>
<td>Kim et al. (2007)(^ {26} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EAE mouse</td>
<td>Intravitreal injection of SRT647, SRT501</td>
<td>Reduced neuronal damage in optic neuritis</td>
</tr>
<tr>
<td></td>
<td>Shindler et al. (2007)(^ {27} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPTP-treated mouse</td>
<td>Intravenous injection of resveratrol</td>
<td>Attenuated neuronal damage in substantia nigra</td>
</tr>
<tr>
<td></td>
<td>Lu et al. (2008)(^ {28} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>Transgenic mouse</td>
<td>Whole-body Sirt1 overexpression</td>
<td>Reduced hepatic inflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td>Pfluger et al. (2006)(^ {29} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Dietary resveratrol</td>
<td>Decreased expression of inflammatory cytokines</td>
</tr>
<tr>
<td></td>
<td>Pearson et al. (2008)(^ {20} )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** apoE\(^ {−/−} \), apolipoprotein E-deficient; DIO, diet-induced obesity; EAE, experimental autoimmune encephalitis model of multiple sclerosis; fa/fa, leptin receptor-deficient obese; IGF-1, insulin-like growth factor 1; ob/ob, leptin-deficient obese; SRT1720, SRT501, SRT647, small-molecule SIRT1 activators; MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), neurotoxin causing permanent symptoms of Parkinson’s disease; p25 (activator of the protein kinase cdk 5) transgenic, mouse model of Alzheimer’s disease.
glucose levels within 2 weeks of dosing, with the effect persisting over 10 weeks of dosing. After 10 weeks of treatment, plasma insulin levels decreased by 50% compared to vehicle-treated controls. Glucose and insulin tolerance tests revealed positive effects of SRT1720, similar to those achieved when DIO mice were treated with rosiglitazone, a drug prescribed widely to lower blood glucose levels in patients with type 2 diabetes. SRT1720 equally improved glucose homeostasis in genetically obese, insulin-resistant Lep≤/≤mice, decreasing plasma glucose levels to near normal levels after 1 week of daily oral dosing. Therapeutic efficacy of SRT1720 was additionally examined by using genetically obese Zucker fa/fa rats, one of the most commonly used models of insulin resistance. Similar to the obese mice, treatment of fa/fa rats with SRT1720 (100 mg/kg) favorably affected blood glucose and insulin levels. Additional studies using the gold-standard hyperinsulinemic-euglycemic clamp technique confirmed that SRT1720 improved insulin sensitivity in obese fa/fa rats. Milne et al. also showed limited data regarding the effectiveness of SRT501, a reformulated version of resveratrol with improved bioavailability (11%) but a shorter half-life than SRT1720 (1 hour versus 5 hours). Oral dosing with SRT501 (500–1000 mg/kg) for 2–4 weeks decreased plasma fasting glucose levels to near normal levels in DIO and Lep≤/≤mice.

SIRT1 ACTIVATION: MECHANISMS OF ACTION

Kinetic studies using recombinant SIRT1 suggest that polyphenolics such as resveratrol increase SIRT1 deacetylation activity by decreasing the Michaelis constant (K_m) of SIRT1 for acetylated substrate. Studies by Milne et al. reveal a similar mechanism for the novel SIRT1 activators SRT1460, SRT2183, and SRT1720. By testing each compound together with resveratrol, they determined that these structurally diverse compounds appear to bind to a single allosteric site on the SIRT1-substrate complex. Analyses of serial SIRT1 truncations mapped the putative allosteric binding region to amino acids 183–225 of the enzyme. It has been proposed that binding of compounds to SIRT1 in vivo not only activates the enzyme, it may also alter its specificity for distinct acetylated substrates. Another way to alter SIRT1 activity is through changing the cellular concentration or activity of nicotinamide, an end-product inhibitor of SIRT1 (Figure 1). A reduction in nicotinamide activity by competitive inhibition with isonicotinamide has been shown to activate Sir2, the yeast orthologue of SIRT1. Accordingly, in mice, supplemental nicotinamide (500 mg/kg diet) elicited some effects opposite of SIRT1 activation: it increased body weight, energy intake, fat mass, and fasting blood glucose levels.

POTENTIAL TOXICITY OF SIRT1 ACTIVATORS

Limited data exist regarding the toxicity and safety of SIRT1 activators. In rats, oral administration of trans-resveratrol at 20 mg/kg for 28 days produced no harmful effects as assessed by growth, hematology, clinical chemistry, and histopathology. This resveratrol dose is estimated to be equivalent to 1000 times the amount that would be consumed by a person drinking one glass of red wine daily. When substantially higher amounts of trans-resveratrol (300, 1000, and 3000 mg daily for 28 days) were tested in rats, the two highest doses caused kidney damage. In mice, chronic consumption of a modest dose of resveratrol (2400 mg/kg diet), starting at 1 year of age, produced no obvious detrimental effects, as assessed by postmortem histopathological assessments. However, a pilot study revealed that when mice consumed larger doses of resveratrol (18,000 mg/kg diet), five of six animals died within 3–4 months. In humans, ingestion of a single dose of resveratrol (0.5, 1, 2.5, or 5 g; 10 subjects per group) was well tolerated without any severe adverse clinical, biochemical, or hematological events. Studies of chronic resveratrol administration with more subjects are needed to adequately assess the safety profile of resveratrol in humans. Moreover, the optimal degree of SIRT1 activation, especially with the more potent SIRT1 activators, will need to be defined, as illustrated by a recent study of transgenic mice overexpressing heart-specific Sir1. Moderate Sirt1 overexpression (7.5-fold) made the heart more resistant to in vivo oxidative stress and apoptosis, whereas higher overexpression (12.5-fold) increased apoptosis and hypertrophy and decreased cardiac function.

CONCLUSION

In the next two decades, the projected number of individuals in the United States aged 65 years or older will double from 35 million to 71 million, increasing from 12% to roughly 20% of the population. This rapid demographic shift will markedly increase the prevalence of age-related disorders including diabetes, cardiovascular disease, neurodegenerative diseases, and inflammation. Not surprisingly, the search for interventions that can alleviate age-related health problems is rapidly accelerating. Although caloric restriction remains the optimal intervention, it is not acceptable to many and is not a viable option for the old frail elderly, those with disease, or the morbidly obese. Caloric restriction mimetics, such as resveratrol and SIRT1-activating compounds, show much therapeutic promise in animal studies but will need extensive evaluation in clinical trials before they can be recommended for human use. Until then, let’s continue to enjoy our wine.
REFERENCES


Copyright of Nutrition Reviews is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.